Short Communication

Effects of Vitamin/Mineral Supplementation on the Proliferation of Esophageal Squamous Epithelium in Linxian, China[1]

Madhusudan Rao, Fu-Sheng Liu, Sanford M. Dawsey,[2] Kan Yang, Martin Lipkin, Jun-Yao Li, Philip R. Taylor, Bing Li, William J. Blot, Guo-Qing Wang, Klaus J. Lewin, Yu Yu, and Chung S. Yang

National Cancer Institute, Bethesda, Maryland 20892 [M. R., S. M. D., P. R. T., W. J. B.]; Cancer Institute, Chinese Academy of Medical Sciences, Beijing, People's Republic of China [F. S. L., J. Y. L., B. L., G-Q. W., Y. Y.]; Memorial Sloan-Kettering Cancer Center, New York, New York 10021 [K. Y., M. L.]; University of California at Los Angeles, Los Angeles, California 90024 [K. J. L.]; and Rutgers University, Piscataway, New Jersey 08855 [C. S. Y.]

Abstract

Abnormalities of epithelial proliferation have been proposed as an early step in gastrointestinal carcinogenesis. To determine whether micronutrient supplementation may reduce squamous epithelial proliferation in the esophagus, we evaluated proliferation in subjects participating in a randomized nutrition intervention trial in Linxian, China, where esophageal cancer rates are among the highest in the world. After 30 months of intervention involving daily supplementation with multiple vitamins and minerals, an endoscopic survey was performed and squamous biopsies from 512 subjects were labeled with tritiated thymidine and autoradiographed. Analysis showed no treatment effect on the overall amount of squamous epithelial proliferation measured by the total labeling index. However, a measure of the vertical distribution of labeled cells showed lower values with supplementation: a 14% reduction in all subjects ($P = 0.29$), and a 29% reduction in nonsmokers ($P = 0.03$). These results suggest a potential modest benefit for short-term intervention with multiple vitamins and minerals on squamous epithelial cell proliferation of the esophagus in this high-risk population.

Introduction

Epithelial cell proliferation is an active area of research in gastrointestinal cancer. Increased proliferation and an expanded distribution of proliferating cells have been shown in a variety of predisposing conditions and precursor lesions throughout the alimentary tract (1).

Linxian, China, has some of the highest rates of esophageal cancer in the world (2), and there is suspicion that the population's chronic deficiencies of multiple nutrients may be etiologically involved (3, 4). An endoscopic survey conducted among Linxian subjects participating in a prospective randomized nutrition intervention trial gave us an opportunity to examine whether the proliferative activity of esophageal squamous epithelium in this high-risk population was affected by 30 months of daily supplementation with multiple vitamins and minerals.

Materials and Methods

Three thousand three hundred eighteen adult Linxian residents who had cytological evidence of esophageal dysplasia in a 1983 balloon cytology screening examination (5) but no history of cancer were enrolled in the Dysplasia Trial, a randomized, double-blind, placebo-controlled, two-arm nutrition intervention trial (6, 7). Active intervention, consisting of two multivitamin, multimineral tablets (Centrum; Lederle Laboratories, Inc.) and one betacarotene capsule (15 mg as Solatene; Hoffmann-La Roche, Inc.) daily or matched placebos, began on May 1, 1985. Daily doses of the active pills, given in Table 1, were typically two to three times U.S. Recommended Daily Allowances.

In November and December of 1987, after 30 months of active intervention, all Dysplasia Trial subjects with a 1983 cytology diagnosis of Dysplasia 2 (high-grade) and every fifth subject with a 1983 cytology diagnosis of Dysplasia 1 (low-grade) were invited to participate in an endoscopic survey, and 833 subjects were endoscoped (8). The number of subjects excluded from eligibility for the endoscopic examinations because of death or incident cancer, the refusal rates among eligibles, and the prevalence of dysphagia in the endoscoped subjects did not differ significantly by treatment group. Six hundred eighty-five of the endoscoped subjects had a 2.8-mm biopsy taken from a standard site in the middle third of the esophagus. These biopsies were incubated for 1 h in tritiated thymidine solution (Eagle's basic salt solution with 10% fetal calf serum and 5 μCi of tritiated thymidine) at 37°C in a 95% oxygen atmosphere, fixed in 95% ethanol, and processed into paraffin blocks. Adjacent sections from each block were used for routine histology and autoradiography.

All histological and autoradiographic readings were made without knowledge of the subjects' treatment group assignments. The biopsies were classified into five histological categories: normal; acanthosis; esophagitis; squamous dysplasia; and squamous cancer, as previously defined (8, 9).

Autoradiography was performed as previously described (10). A cell was considered labeled if at least five black grains were seen over the nucleus. The flat regions of epithelium between oriented papillae were counted in a seg-

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[2] To whom requests for reprints should be addressed, at Cancer Prevention Studies Branch, Division of Cancer Prevention and Control, National Cancer Institute, Executive Plaza North, Room 211, 9000 Rockville Pike, Bethesda, MD 20892.

Table 1 Daily doses of micronutrients in the supplements of the Dysplasia Trial, Linxian, China[a]

Vitamin/mineral	Compound	Dose
	Acetate	
	dl-alpha tocopheryl acetate	
	Ascorbic acid	
	Thiamine mononitrate	
	Riboflavin	
	Pyridoxine HCL	
	Cyanocobalamin	
	Calcium pantothenate	
	Dibasic calcium phosphate	
	Diabasic calcium phosphate	
	Potassium iodide	
	Ferrous fumarate	
	Magnesium oxide	
	Cupric oxide	
	Manganese sulfate	
	Potassium chloride	
	Potassium chloride	
	Chromium chloride	
	Sodium molybdate	
	Sodium selenate	
	Zinc sulfate	

[a] Participants received 2 multivitamin, multimineral tablets (Centrum; Lederle Laboratories, Inc.) and 1 betacarotene capsule (Solatene; Hoffmann-LaRoche, Inc.) or matching placebos daily.
[b] IU, international units.

ment 200 cells long and 10 cell layers thick starting at the base of the epithelium. The numbers of labeled and unlabeled cells in each cell layer were recorded.

Two proliferation variables were derived from the cell count data: the total labeling index (TLI)[3] (total labeled cells/total cells counted) was our measure of the amount of proliferation, and the proportion of labeled cells found in cell layers 4 through 10 (LF4+) (labeled cells in cell layers 4–10/total labeled cells) was our measure of the vertical distribution of proliferation in each biopsy (11).

For treatment group comparisons, the analytic group included all 512 subjects (259 receiving active supplements and 253 receiving placebo pills) who had a biopsy with satisfactory histological and radiolabeling data. Statistical analysis, performed with the SAS statistical package (12), included unpaired *t*-tests, χ^2 tests and multivariate linear regression analysis.

Results

The median age of the 512 subjects was 57 years (range, 36–74 years); 42% were men, 24% were smokers, and 55% had a 1983 cytology diagnosis of Dysplasia 2. There were no significant differences by treatment group among the endoscoped subjects for any of these characteristics.

Pill disappearance rates, a measure of compliance, were equal in both treatment groups throughout the first 30 months of the Dysplasia Trial, with over 95% of pills reportedly consumed (6). High compliance was also indicated by quarterly biochemical assessments of a sample of the total Dysplasia Trial population which showed significant improvements in blood levels of retinol, riboflavin, ascorbic acid, and betacarotene in the active treatment group throughout the intervention period (6).

Table 2 shows the mean values of the two proliferation variables, by treatment group, with stratifications by age, gender, smoking status, and 1983 cytology grade. There were no significant differences between treatment groups in the mean values of the TLI, in all subjects or in any of the subgroups examined.

The variable reflecting the vertical distribution of proliferating cells (LF4+) had a 14% lower mean value in the total treatment group (3.34%) than in the placebo group (3.86%), but this difference was not statistically significant ($P = 0.29$). The stratifications showed a difference in treatment effect by smoking status: smokers had a higher mean value in the treatment group than in the placebo group, while nonsmokers showed the opposite result. Among nonsmokers, the mean LF4+ value was 29% lower in the treated group (3.08%) than in the placebo group (4.31%), a statistically significant difference ($P = 0.03$). The treatment effect among nonsmokers was similar for males and females. The stratifications also showed a 24% lower mean LF4+ value in the treated group than in the placebo group among subjects with an initial cytology grade of Dysplasia 2 ($P = 0.16$).

The distribution of histological diagnoses was similar in both treatment groups (8), and adjustment for histological diagnoses did not alter the proliferation results.

Discussion

Our analysis showed no effect of 30 months of daily supplementation with multiple vitamins and minerals on the overall amount of squamous cell proliferation measured by the total labeling index. A measure of the vertical distribution of labeled cells, however, showed lower values with supplementation. The overall reduction was modest (14%; $P = 0.29$), but a larger and significant reduction was seen in nonsmokers (29%; $P = 0.03$).

The observation that the proliferation variable reflecting the vertical distribution of labeled cells showed more effect of treatment than the total labeling index may indicate that the distribution of labeled cells is more sensitive to nutritional changes than the total number of such cells or that distributional changes precede changes in the overall amount of proliferation.

Several factors may have limited our ability to observe significant treatment effects. The nutrients may have been given for an insufficient length of time or in insufficient doses for an effect to be seen, or effects of some nutrients may have been balanced and masked by opposite effects of others in this multiple vitamin/mineral supplementation. A single biopsy may have been insufficient sampling to correctly reflect each subjects's relevant proliferation status (such sampling would correctly classify subjects only if epithelial proliferation is relatively uniform, a "field effect," throughout each esophagus; the variability of esophageal epithelial proliferation within individuals in this population has not yet been examined). Counting cells only in the flat areas of the epithelium between the papillae may have missed some important proliferation information in the papillary regions. Finally, although the sample size was relatively large for this type of investigation, it was not sufficient to detect small reductions in mean proliferative activity.

[3] The abbreviations used are: TLI, total labeling index; LF4+, labeled cell fraction four plus.

Table 2 Mean proliferation values, by treatment group, stratified by age, gender, smoking status, and 1983 cytology diagnosis

Subject group	No. of Subjects		TLI[a]			LF4[b]		
	Placebo	Treatment	Placebo	Treatment	% Δ[c]	Placebo	Treatment	% Δ
All subjects	253	259					3.34	
Age (yr)								
<57	129	125	3.54	3.54	0.0	3.88	3.42	−11.9
≥57	124	134	3.54	3.55	+0.3	3.84	3.26	−15.1
Sex								
Male	98	115	3.41	3.45	+1.2	3.00	3.46	+15.3
Female	155	144	3.63	3.63	0.0	4.40	3.24	−26.4
Smoking status[d]								
Smoker	60	61	3.46	3.43	−0.9	2.55	3.87	+51.8
Nonsmoker	191	196	3.56	3.58	+0.6	4.31	3.08	−28.5[e]
Male	37	53	3.30	3.48	+5.5	3.82	3.03	−20.7
Female	154	143	3.62	3.62	0.0	4.43	3.10	−30.0
1983 Cytology diagnosis								
Dysplasia 1	114	116	3.48	3.59	+3.2	3.35	3.46	+3.3
Dysplasia 2	139	143	3.59	3.52	−1.9	4.27	3.24	−24.1

[a] Total labeling index.
[b] Fraction of labeled cells in layers 4–10.
[c] (Treatment value − Placebo value) × 100 ÷ Placebo value.
[d] All known smokers were men. The smoking status of two men and two women was not known.
[e] $P < 0.05$.

During the 6-year Dysplasia Trial intervention, there was a 6% lower risk of developing esophageal cancer and a 16% lower risk of esophageal cancer death among subjects in the treatment group than among those in the placebo group, but neither of these results was statistically significant (7). In two endoscopic surveys of Dysplasia Trial subjects, there was a nonsignificant 26% lower prevalence of esophageal dysplasia and cancer in the treatment group after 30 months of supplementation, but there was essentially no difference in the prevalence of these lesions by treatment group after the full 6 years of intervention (8). As with the current analysis, these related studies suggest but do not establish a potential benefit for supplementation with multiple vitamins and minerals in this population.

Only one previous study has evaluated the effect of a nutritional supplement on esophageal squamous proliferation in a prospective, randomized clinical trial (13). That study, also conducted in a high-risk Chinese population, reported no effect of 11 months of high-dose oral calcium supplementation on the total labeling index or the distribution of proliferation.

In summary, we examined the effect of 30 months of daily supplementation with multiple vitamins and minerals on squamous epithelial cell proliferation of the esophagus in subjects from a population with a high risk for developing esophageal cancer. We found no significant difference in the overall amount of proliferation between the treated and placebo groups, but more of the proliferating cells were found in the lower epithelial layers in the supplemented group, suggesting a potential benefit for such supplements. Work is in progress to see if our cell proliferation results will correlate with subsequent development of squamous esophageal cancer.

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